STUDIES ON THE EFFECT OF PROBIOTIC AND PHYTOGENIC FEED ADDITIVES ON HEALTH AND GROWTH PERFORMANCE OF BROILER CHICKENS AT HIGH ALTITUDE

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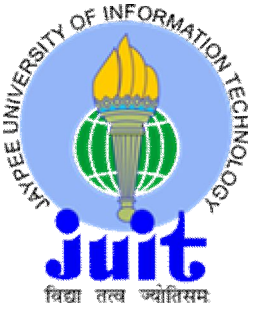

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Introduction

Ladakh sector is a strategic place for India and large number of armed force are deployed in this region which required continues supply of animal origin fresh food for health and operational requirements. Livestock plays significant part in fulfilling the nutritional requirements of Indian troops by providing nutritional security (meat and milk). Livestock also help in the socio-economic upliftment of local people of Leh-Ladakh. However, the harsh climatic conditions of trans-Himalayan Ladakh lead to poor productive and poor reproductive performance of livestock animals in Leh-Ladakh and this led to the poor availability of fresh animal origin food to locals and troops deployed in this region. As most of the land in Ladakh sector is barren and uncultivated, therefore, there is a huge shortage of nutritious animal feed resources which limits animal husbandry practices in this region [1]. In the last twenty years, broiler poultry industry in India attains very stunning progress. However, the same is not true for Ladakh region wherein poultry industry is virtually nonexistence. Both productive and reproductive performance of broiler chickens is very poor at high altitude as compared to their performance at low altitudes. A major constraint for the difference in the growth could be attributed to stressful environmental conditions of this region which are characterised by extreme temperature variations (from +35°C to -35°C), hypobaric hypoxia, high UV radiations, low humidity, and scarcity of fodders. These types of adverse climatic conditions does not suit the natural habitat of poultry birds as it contributes to oxidative stress in poultry birds which hinders their growth rate and optimum production [2].

The low temperature at high altitude often leads to increase in basal metabolic rate which requires more oxygen, however, reduced partial pressure of oxygen at high altitude conflict with higher oxygen demand which inevitably results in higher mortality in birds due to ascites and pulmonary hypertension [3]. Fall in the immunity in birds under hypoxic conditions also makes them susceptible to numerous microbial diseases, including coccidiosis [4]. Moreover, birds are also infected with coccidiosis and other microbial diseases at high altitude due to falling in the immune system. Further factors which have influenced poultry production are the unavailability of suitable poultry feed. Still so for no special feed has been developed which can meet nutritional requirements of broiler birds at high altitude. Therefore, available commercial broiler feed has no nutritional composition to meet the

maintenance and productive requirement of birds at high altitude. Besides, the region is cut off from rest of the country for up to 6-7 months in a year due to which it is very difficult to procure day old chicks and readymade poultry feed from other parts of the country during the winter season. Due to all these reasons, the poultry industry is not more successful in Ladakh and has been an underdeveloped venture [2].

Harsh environmental conditions of high altitude are not appropriate for the growth of local poultry breed as well as for plain area breeds. At high altitude, there is a scarcity of appropriate high-yielding variety of layers and broiler breeds those are adapted to regional harsh climatic conditions. There are no available poultry breeds/strains which are suitable to this high altitude region. Therefore, our institute (DIHAR DRDO) is working on development of new strains/breeds suitable for this region. So for, White Rock, Red Cornish, RIR cross-bred, Black Rock etc. are found suitable. However, detailed studies are going on which may reveal their suitability for cross breeding, pure breeding for economic weight gain, survivability etc. New broiler breeds could be identified by carrying out experimental field trials at high altitude and broiler strains which are adaptable to hypoxic conditions might have better growth rate with less susceptibility to high altitude stress. Moreover, there is a need to develop a suitable feed for poultry birds having stress-ameliorating properties. This can be achieved by developing suitable feed additives. Furthermore, development of suitable feed formulation using different feed resources through exploring their efficacy in poultry nutrition could be a better alternative for improvement in the performance of poultry birds at high altitude. However, the role of probiotics and phytogenics on the performance of poultry birds at high altitude cold desert still remains unexplored. Therefore, considering the above-mentioned problems, the present study has been conducted with the following objectives:

1. To study the effect of probiotic on growth performance and survivability of different broiler chickens at high altitude.
2. To standardize extraction and identification of flavonoids and total phenolic contents in *Rhodiola imbricata* roots, *Hippophae rhamnoides* fruits, *Salix alba leaves, and Prunus armeniaca* seeds and their *in-vitro* evaluation of antioxidant and cytoprotective properties.

3. To evaluate the efficacy of phytoextracts on physio-biochemical indices, growth performance, survivability of broiler chicken and cost economics of their rearing at high altitude.
4. To formulate the phytogenic feed additive for broiler chicken and a comparative study on their efficacy with other commercially available feed additives.

Objective 1: To study the effect of probiotic on growth performance and survivability of different broiler chickens at high altitude.

Material and methods

The study was conducted in two experimental trials which were carried out under deep litter system in the solar poultry house having a stacking density of 0.80 square feet per bird in 4x2 feet of pen size (10 birds/pen). In the first trial, growth performance of different broiler strains was compared to find out suitable broiler strain for high altitude. A total of 150 numbers of one week old chicks (brought from commercial hatchery of plain areas) were randomly selected from three commercially available broiler strains i.e. Vencobb, RIR Cross-bred, and Hubbard (50 chicks from each breed) and divided into 3 groups of 5 replicates with 10 chicks in each replicate as per completely randomized design. All the birds were given the same basal diet. The experiment was conducted from 7 to 35 days of broiler age. All the chickens were weighed individually at every week interval up to 35 days of age. Throughout the experiment, water and feed intake were measured. Feed: gain (FCR) was calculated from the ratio between feed intake and body weight gain of chick.

Thereafter, a second trial was conducted where probiotic (BiobloomT, M/S Zydus Animal Health Limited, India) mixed basal diet was supplemented to the best-performing broiler strain (among the above-mentioned three strains). A total of 150 one week old commercial broiler chicks were randomly assigned to 3 groups in 5 replicates (10 chicks in each replicate) having 50 birds in each group. Basal diet was given to control group birds whereas the two treatment groups T1 and T2 were supplemented with probiotic @ 9 gm/kg feed (0.45×10^9 CFU of *Bacillus coagulans* and 13.5×10^9 CFU of *Saccharomyces cerevisiae)* and @ 18 gm/kg feed (0.9×10^9 CFU of *Bacillus coagulans* and 27×10^9 CFU of *Saccharomyces* cerevisiae) respectively, along with the basal diet. Each gram of probiotic contains 50×10^6 CFU of *Bacillus coagulans* and 1500×10^6 CFU of *Saccharomyces cerevisiae*. Birds dead during both trials were examined for ascites and coccidiosis on post-mortem examination. Statistical analysis was performed with SPSS software (version 17.0) as

per the standard procedure of one-way ANOVA. Results were expressed as a mean ± standard error. The significant statistical difference was assumed at $P<0.05$.

Result

In the first experimental trial, body weight gain (BWG) was significantly ($P<0.05$) increased in RIR cross-bred birds as compared to other two strains. No differences were observed in BWG among Vencobb and Hubbard strain. Feed intake was significantly higher ($P<0.05$) in Hubbard birds followed by RIR cross-bred and Vencobb. Moreover, feed: gain (FCR) value was improved significantly ($P<0.05$) in RIR cross-bred followed by Vencobb and Hubbard. Water intake did not differ among the groups throughout the experiment. Collectively, BWG and FCR data illustrated that RIR cross-bred had improved growth performance in comparison to Vencobb and Hubbard strains at high altitude. The fall in the growth performance of Vencobb and Hubbard strain birds might possibly be due to their high metabolic rate as these birds are fast growing which requires more oxygen at tissue level to sustain fast growth [5]. The mortality rate was recorded highest (30%) in Hubbard strain followed by Vencobb (22%) and RIR cross-bred (16%). Post-mortem examination of birds revealed 14%, 10%, and 6% mortality induced from ascites and 10%, 8%, and 4% mortality induced from coccidiosis in Hubbard, Vencobb, and RIR cross-bred, respectively. RIR cross-bred exhibited less mortality from both ascites and coccidiosis which might be due to their hardiness nature and less metabolic rate as compared to fast-growing Vencobb and Hubbard [6]. Hence, for the rearing purpose, the RIR cross-bred strain may be helpful at high altitude.

In the second experimental trial, we examined the effect of dietary supplementation of probiotic on growth performance of RIR cross-bred birds (as it performed best in the experimental trial I). Interestingly, we did not observe any beneficial effect of probiotic on BWG and FCR of treatment groups as compared to control group might be due to the inappropriate type of microbes in the probiotic or inadequate amount of *Bacillus coagulans* and *Saccharomyces cerevisiae* strain that was used [7]. However, the dose of microbes in the used probiotic was much excessive than the dose of plain areas. Therefore, at high altitude different sort of microbes might be appropriate for poultry birds. Furthermore, the mortality rate was recorded highest (30%) in control group followed by T1 (20%) and T2 (16%) groups. Post-mortem examination of birds revealed 10%, 8%, and 6% mortality induced from ascites and 16%, 6%, and 4% mortality induced from coccidiosis in control, T1, and T2 groups, respectively. At the end of the experiment, economics were calculated based on

rearing cost of 50 no. of birds. For the experiment, the cost of probiotic was included along with the feed cost whereas other expenditure remained constant. Probiotic supplementation reduces the mortality in birds as compared to control group and this result increased in the net return.

Conclusion

In conclusion, by developing suitable broiler strains having more adaptability under hypoxic conditions at high altitude would be beneficial to overcome unsatisfactory growth performance at high altitude. Among the three broiler strains, RIR cross-bred has shown better growth and survivability, and hence would be suitable for high altitude. Due to less mortality in probiotic-supplemented group, net returns revealed a higher profit. Therefore, to save the loss due to high mortality, dietary supplementation of probiotic as a feed additive in broilers diet could be a valuable source. However, before the selection of probiotics, extra knowledge is required on dose, gut microflora and probiotic efficacies of microfloral species in poultry a high altitude.

Objective 2: To standardize extraction and identification of flavonoids and total phenolic contents in *Rhodiola imbricata* roots, *Hippophae rhamnoides* fruits, *Salix alba* leaves, and *Prunus armeniaca* seeds and their *in-vitro* evaluation of antioxidant and cytoprotective properties.

Material and methods

For this study, we screened the literature reported by previous co-workers [8, 9, 10] about the high altitude trans-Himalayan flora and we selected four native plants viz. *Rhodiola imbricata* (Rhodiola), *Hippophae rhamnoides* (seabuckthorn), *Salix alba* (willow), and Prunus *armeniaca* (apricot) based on their medicinal properties and there potential as feeding material in livestock rearing for the preparation of phytogenic feed additive. *Rhodiola imbricata* roots were collected from the trans-Himalayan region (Chang-La Top, altitude = 5330 m above mean sea level, Ladakh), *Hippophae rhamnoides* fruits and *Prunus armeniaca* seeds were collected commercially from Leh market whereas *Salix alba* leaves were collected from DIHAR campus. After collection, all the samples were washed thoroughly, shed dried at room temperature and ground to obtain a fine homogeneous powder. Thereafter, powdered samples of *R. imbricata* roots, *Hippophae rhamnoides* fruit, *Salix alba* leaves, and *Prunus armeniaca* seeds were extracted with different solvents (hydro-ethanolic (40:60), aqueous, methanol, and n-hexane) in soxhlet apparatus for standardization of extraction method.

The standardized extract of each plant was phytochemical characterized for total phenolics [11], flavonoids [12], and carotenoids [13] contents, and also evaluated for total antioxidant capacity (TAC) [14], DPPH radical scavenging activity [15] and ABTS radical scavenging activity [16]. Furthermore, quantification of extracts for phytomolecules was carried by reverse phase HPLC (Shimadzu LC10ATvp) through a C18 column (250 mm × 4.60 mm, 5 µm) using SPD-10 Avp UV-Visible detector.

To determine the antioxidant and cytoprotective activities of different extracts their efficacy was first evaluated *in vitro* in chicken peripheral blood lymphocytes (PBL). Blood samples were collected from the wing vein of healthy broiler chickens and peripheral blood mononuclear cells (PBMC) were isolated using Histopaque-1077. Adherent (monocytes) and non-adherent (lymphocytes) cells were separated by incubating PBMC at 41^0C for 45 min using plastic adherence technique described by Gupta et al. [17]. Separated PBL were adjusted to concentration of 1×10^4 cells/mL in RPMI-1640 medium supplemented with 10% FBS, 100 U/mL penicillin and 100 µg/mL streptomycin. Thereafter, PBL suspension (100 µL/ well) was cultured in 96-well microtiter plate with 100 µL/well of different dose concentrations of *R. imbricata* extract, *H. rhamnoides* extract, *S. alba* extract, and *P. armeniaca* extract (100, 200, 400, 800 ng/mL, and 1, 2, 4, 8, 50, 100, 200, 400 µg/mL), 1 µg/mL of concanavalin A as positive control, and medium as negative control, at 41^0C in a 5 % CO_2 incubator for 24 hrs. The cellular proliferative activity of the extracts was measured with MTT assay described by Mosamann [18]. Cytoprotective activity of extract against the toxic effect of H_2O_2 was also determined by MTT assay.

Result

In soxhlet extraction, extract yield was recorded maximum in a hydro-ethanolic solvent (30.8 %) in *R. imbricata* root extract followed by aqueous (23.87%), methanol (18.3%) and n-hexane (2.75%). For *H. rhamnoides*, the maximum yield of extract was noticed in aqueous solvent (32.7%) followed by hydro-ethanol (28.42%), methanol (25.08%) and n-hexane (16.47%). Similarly, *S. alba* leaves exhibits maximum extraction yield in aqueous solvent (37.02%), followed by hydro-ethanol (31.07%), methanol (30.68%), and n-hexane (3.38%). In contrast, *P. armeniaca* seeds exhibits maximum extraction yield in aqueous solvent (37.41%), followed by n-hexane (36.09%), methanol (33.03%), and hydro-ethanol (31.8%).

Thereafter, the extracts with maximum yield in particular solvent (hydro-ethanolic extract of *R.imbricata,* aqueous extract of *H. rhamnoides, S. alba* and *P. armeniaca* respectively) were phytochemically characterized for the presence of different bioactive phytomolecules. Free radical scavenging capacity of different extracts was determined by DPPH and ABTS assay and all the extracts were found to scavenge the DPPH and ABTS radical in a dose-dependent manner at a concentration of 20 to 100 µg/mL as similar to positive control ascorbic acid.

TAC of different phytoextracts were estimated by FRAP assay and it was recorded to be 473.41 ± 18.47 µM Fe (II)/g of extract in hydro-ethanolic extract of *R. imbricata,* 425.54 ± 16.14 µM Fe (II)/g of extract in aqueous extract of *Hippophae rhamnoides,* 451.68 ± 19.31 µM Fe (II)/g of extract in aqueous extract of *S. alba* and 409.78 ± 16.61 µM Fe (II)/g of extract in aqueous extract of *P. armeniaca* respectively. The concentrations of total phenols in different phytoextract was recorded to be 219.72 ± 1.71 mg GAE/g of extract in *R. imbricata,* 76.28 ± 3.25 mg GAE/g of extract in *H. rhamnoides,* 234.14 ± 2.47 mg GAE/g of extract in *S. alba,* and 0.63 ± 0.22 mg GAE/g of extract in *P. armeniaca,* respectively.

Total flavonoids concentration in different phytoextract was recorded to be 184.05 ± 2.14 mg QE/g of extract in *R. imbricata,* 35.14 ± 2.18 mg QE/g of extract in *H. rhamnoides,* 86.32 ± 1.81 mg QE/g of extract in *S. alba,* and 0.40 ± 0.14 mg QE/g of extract in *P. armeniaca,* respectively whereas the carotenoids content was recorded to be 0.17 ± 0.04 mg/100 g extract in *R. imbricata,* 4.19 ± 0.70 mg/100 g extract in *H. rhamnoides,* 2.87 ± 0.26 mg/100 g extract in *S. alba,* and 0.68 ± 0.31 mg/100 g extract in *P. armeniaca,* respectively.

HPLC analysis of *R. imbricata* extract confirmed the presence of p-tyrosol and salidroside at a concentration of 232.40 and 191.01 µg in 1 mg of extract respectively. Similarly, HPLC analysis of *H. rhamnoides* extract revealed the presence of gallic acid and kaempferol at a concentration of 210.24 and 26.52 µg in 1 mg of extract. Furthermore, HPLC analysis of *Salix alba* leave extract confirmed the presence of salicin and caffeic acid at a concentration of 790.18 and 18.11 µg in 1 mg of extract, respectively. Moreover, HPLC analysis of *P. armeniaca* extract revealed the presence of (+)-catechins at a concentration of 320.40 µg in 1 mg of aqueous extract.

Hydro-ethanolic extract of *R.imbricata* stimulated the proliferation of chicken PBL at all tested dose concentration in between 800 ng/mL to 400 µg/mL in a dose-dependent manner compared with the untreated control cells and also protect the cells from H_2O_2

induced oxidative stress at all tested dose concentrations between 800 ng/mL to 400 µg/mL as compared with H_2O_2 stimulated cells. Similarly, aqueous extract of *H. rhamnoides* stimulated the PBL proliferation at all tested concentrations (100 ng/mL-400 µg/mL) in a dose-dependent manner compared with the untreated control cells and reduced the cytotoxicity induced by H_2O_2 at a dose concentration of 100 ng/mL-400 µg/mL compared with H_2O_2 stimulated cells.

In the case of aqueous extract of *S. alba* leaves a dose-dependent increase in the lymphocyte proliferation was recorded at the concentration of 400 ng/mL-400 µg/mL compared with untreated control cells and moreover, treatment of *S. alba* extract with lymphocytes reduced the H_2O_2 induced cytotoxicity at dose concentration of 400 ng/mL-400 µg/mL compared with H_2O_2 stimulated cells. Furthermore, aqueous extract of *P. armeniaca* also found to stimulate lymphocyte proliferation in a dose-dependent manner at 100 ng/mL-400 µg/mL concentrations compared with untreated control cells and reduced the H_2O_2 induced oxidative stress in lymphocytes at 100 ng/mL-50 µg/mL dose concentration compared to H_2O_2 stimulated cells.

Conclusion

Hydro-ethanolic extract of *Rhodiola imbricata* roots and aqueous extract of *Hippophae rhamnoides* fruits, S*alix alba* leaves and *Prunus armeniaca* seeds were phytochemically characterized which revealed rich in polyphenols, flavonoids, and carotenoids content. Moreover, *in-vitro* studies indicated enhanced antioxidative and cytoprotective activities of these phytoextracts on chicken peripheral blood lymphocytes. Therefore, considering the pharmacological antioxidant properties of these four extracts their *in-vivo* efficacy might be evaluated on different physio-biochemical indices and growth performance of broiler chickens.

Objective 3: To evaluate the efficacy of phytoextracts on physio-biochemical indices, growth performance, survivability of broiler chicken and cost economics of their rearing at high altitude.

Material and methods

The study was conducted in four experimental trials, after taking approval from Institutional Animal Ethics Committee. During each experimental trial, 105 one day old RIR cross-bred broiler chicks were distributed into 7 experimental groups as per complete randomized design. There were 3 replications per experimental group with 5 chickens per replicate pen.

Experimental groups included control (fed the basal diet), and treatments T1, T2, T3, T4, T5, and T6 which received an hydro-ethanolic extract of *Rhodiola imbricata* in experiment 1, aqueous extract of *H. rhamnoides* fruits in experiment 2, aqueous extract of *S. alba* leaves in experiment 3, and aqueous extract of *P. armeniaca* seeds in experiment 4 in drinking water at concentration of 100, 150, 200, 300, 400, and 800 mg/kg body weight of chicken respectively, along with the basal diet. The experimental period for each study was 0 to 42 days of broiler chick age. Antibiotics, anticoccidials or any other commercially available vitamin/mineral supplement were not added to drinking water in order to avoid interference. Each chicken was individually weighed at every week interval during each experimental trial. Throughout the experiment, water and feed intake was measured. FCR was calculated from the ratio between feed intake and weight gain by a chick. During each experimental trial post-mortem examination of dead birds was done to find out the cause of death. Economics of each experiment trial was also estimated based on the rearing cost of birds.

During each experimental trial, blood samples were taken at 0, 21, and 42 day for examination of physio-biochemical indices. All blood plasma biochemical parameters including glucose, cholesterol, triglyceride, HDL, LDL, total protein, albumin, creatinine, ALT and AST were analysed with a biochemical semi-auto analyser (BIOTRON BTR-830) using commercial available biochemical kits according to the methodology. Plasma TAC and free radical scavenging activity were analysed by the method described by Benzie and Strain [14] and Brand-Williams et al. [15], respectively. Lipid peroxidation status was estimated by the method proposed by Buege and Aust [19]. The concentration of inflammatory cytokines IL-1, IL-2, and IL-6 were determined using commercially available ELISA kits (Biolegend, San Diego, CA) according to methodology suggested by manufacturer. All statistical analysis was performed with SPSS statistical software package version 17.0 (SPSS, Chicago). Data were analyzed by one-way analysis of variance (ANOVA) using completely randomized design. Values were expressed as a mean ± standard error. $P<0.05$ was assumed to be statistically significant.

Result

In experiment 1, a significant increase in the plasma total protein and globulin concentration was recorded in treatment groups at 42 day and the highest level was recorded in the T5 group (supplemented with hydro-ethanolic extract @ 400 mg/kg). No difference was recorded in albumin concentration. A significant decrease in A/G ratio was noticed in

treatment groups as compared to control group. Level of cholesterol and triglyceride were significantly lower in T2 and T5 groups as compared to control group. Plasma HDL concentration reduced significantly in control group birds as compared to the treatment groups. Among the treatment groups birds in T5 group represents highest HDL concentration. A significant decrease in LDL concentration was recorded in treatment groups as compared to control group. Among the treatment groups, birds in T5 group had lowest LDL concentration. A significant increase in plasma glucose level was observed in control group birds. No differences (P>0.05) were observed in the mean values of creatinine among the experimental groups. AST & ALT levels were significantly reduced in treatment groups compared to the control group. Among the treatment groups, birds in the T5 group represent lowest ALT and AST concentration.

In the experimental trial 2, supplementation of *H. rhamnoides* extract significantly increased the level of plasma total protein, albumin, and globulin in treatment groups as compared to control group. Among the treatment groups, total protein and globulin level were significantly higher in T3 group (supplemented with aqueous extract @ 200 mg/kg) birds at 42 day of age. A significant decrease in A/G ratio was noticed in treatment group birds as compared to control group. Plasma cholesterol and LDL were significantly lower in treatment group birds whereas the HDL level was recorded higher in the treatment group. Among the treatment groups, birds in the T3 group represent highest HDL concentration. No differences were observed in the mean values of triglyceride, creatinine and ALT concentration. AST level was significantly reduced in T3 and T4 group birds as compared to control group.

In the experimental trial 3, supplementation of aqueous extract of *S. alba* leaves increased the concentration of plasma total protein, albumin, and globulin in treatment group birds as compared to control group. Among the treatment groups, birds in T4 (supplemented with aqueous extract @ 300 mg/kg) group represented the highest concentration of total protein and albumin. Moreover, no difference was observed in the level of A/G ratio, creatinine, and ALT among the experimental groups. A significant decreased in the concentration of plasma cholesterol, triglyceride, and LDL was recorded in a T4 group whereas highest level of HDL was recorded in T4 group birds as compared to other experimental groups.

In experiment 4, plasma total protein, albumin and globulin concentration was elevated in treatment groups with supplementation of *P. armeniaca* extract. Among the treatment groups, birds in T3 (supplemented with aqueous extract @ 200 mg/kg) group represented the highest level of total protein. No difference was observed in the level of globulin among the experimental groups at 21 day of age whereas at 42 day of age globulin level was increased in treatment group birds in comparison to control group. Plasma cholesterol and triglyceride concentration were reduced in T3 group birds in comparison to control group. However no differences were observed in the level of HDL, LDL, and creatinine among the groups whereas plasma ALT and AST concentration were reduced in T3 group birds.

In experiment 1, a significantly higher level of TAC and free radical scavenging activity was measured in treatment groups and birds in the T5 group represent the highest TAC and free radical scavenging activity at 42 day. Furthermore, MDA concentration was decreased in the treatment groups and lowest MDA level was recorded in T5 group. Similarly, in experimental trial 2, plasma free radical scavenging activity and TAC was increased in treatment groups as compared to control and highest concentration was recorded in T3 group birds. MDA concentration was recorded significantly ($P<0.05$) lower in treatment groups as compared to control group and among the treatment groups, lowest MDA concentration was recorded in T3 group birds at 42 day.

During the experimental trial 3, supplementation of *S. alba* extract enhanced the free radical scavenging capacity as well TAC in chicken plasma samples. In between treatment groups, birds in T4 group represented highest scavenging activity and TAC. MDA concentration was decreased in treatment group birds as compared to control group and lowest MDA concentration was recorded in T4 group birds throughout the experiment. Furthermore, supplementation of *P. armeniaca* extract in experiment 4 enhanced the free radical scavenging activity and TAC in treatment groups in comparison to control group and highest scavenging activity and TAC was recorded in T3 group birds. MDA level was reduced in treatment groups as compared to control group and among the treatment groups, lowest MDA level was recorded in T3 group birds at 42 day.

In experiment 1, the concentration of inflammatory cytokine IL-1 did not vary among the experimental groups, whereas IL-2 level was found to be significantly higher in T3, T4, T5 and T6 groups as compared to control T1 and T2 group. The concentration of

inflammatory cytokine IL-6 was reduced in T3, T4, T5, and T6 groups as compared to control group at 21 and 42 days. Among the treatment groups, the numerically lowest IL-6 level was recorded in T5 group.

In experimental trial 2, the concentration of IL-1 did not vary among the experimental groups at 21 and 42 day of experiment whereas the level of IL-2 was increased in treatment groups as compared to control group. Among the treatment groups, birds in T3 and T4 groups represent the highest IL-2 level. Furthermore, the concentration of proinflammatory cytokine IL-6 was recorded significantly lower in treatment groups as compared to control group birds. Among the treatment groups, birds in T3 and T4 group had a significantly lower IL-6 level.

In experiment 3, the concentration of IL-1 was recorded significantly lower in T4 group birds as compared to remaining experimental groups whereas; the level of IL-2 was recorded significantly higher in T4 group birds. Furthermore, the concentration of IL-6 was found to be significantly decreased in treatment group birds as compared to control group. Among the treatment groups, birds in the T4 group represented a lowest IL-6 level.

In experimental trial 4, the concentration of IL-1 did not differ among the experimental groups whereas; the level of IL-2 was increased in treatment groups as compared to control group at 21 and 42 days of the experiment. Among the treatment groups birds in T3 and T4 groups represent the highest IL-2 level. Moreover, the level of proinflammatory cytokine IL-6 was significantly reduced in treatment group birds as compared to control group birds.

In experiment 1, supplementation of a hydro-ethanolic extract of *R. imbricata* significantly increased the body weight in treatment group birds as compared to control group and highest body weight was recorded in the T5 group (received hydro-ethanolic extract @ 400 mg/kg) birds followed by T3, T4, T2, T1, and T6. No differences were observed in the mean value of cumulative feed and water intake among the groups. FCR value in T5 group was significantly improved among the groups. Highest mortality rate (13.33%, total 2 birds out of 15) was recorded in the control group birds followed by T1, T3, and T5 (6.67%, total 1 out of 15). Interestingly no mortality was recorded in T2, T4, and T6 groups during the whole experiment. Post-mortem examination of control group birds revealed one mortality induced from ascites and one from coccidiosis whereas single mortality that was observed in T1 group was induced from ascites. Economics of the experiment was also calculated based on the rearing cost of 15 no of birds in each group. The additional cost of the extract was included

with feed cost whereas other expenditure remained constant. *R. imbricata* reduced the mortality rate in treatment group birds and which subsequently results increased in the net return.

During the experimental trial 2, the live body weight of chickens was increased in all treatment groups that received *H. rhamnoides* extract as compared to control group and highest body weight was recorded in T3 group (supplemented with *H. rhamnoides* extract @ 200 mg/kg body weight of chicken) birds followed by T1, T5, T2, T4, and T6. No differences were observed in cumulative feed and water intake of birds among different experimental groups. FCR value was improved in T3 group birds. Highest mortality rate (26.67%, total 4 birds out of 15) was recorded in the control group birds followed by T1, T2, T4, T5 and T6 (13.30%, total 2 out of 15), followed by T3 (6.67%, total 1 out of 15). Post-mortem examination revealed 13.30%, 6.67%, 6.67%, 0%, 0%, 0%, and 6.67% mortality in birds induced from ascites and 6.67%, 0%, 6.67%, 0%, 0%, 6.67%, and 0% mortality induced from coccidiosis in control, T1, T2, T3, T4, T5, and T6 groups, respectively. *H. rhamnoides* reduced the mortality rate in treatment group birds as compared to control group and which results increased in the net return.

In experiment 3, birds in T4 group had significantly ($P<0.05$) higher live body weight as compared to control and other treatment groups at 21 days of age. Moreover, no differences were observed in live body weight of chickens among the control and other treatment groups at 21 days of age. At 42 day of age, the live body weight of chickens was found to be significantly increased in T4 group birds (supplemented with *S. alba* extract @ 300 mg/kg body weight of chicken) followed by T3 group as compared to control and other treatment groups. FCR value was improved in T4 group birds. Highest mortality rate (53.33%, total 8 birds out of 15) was recorded in the control group birds followed by T2 and T6 (33.33%, total 5 out of 15), followed by T3 (26.67%, total 4 out of 15), and followed by T1, T4, and T5 groups (20%, total 3 out of 15). Post-mortem examination revealed 26.67%, 0%, 6.67%, 6.67%, 6.67%, 0%, and 0% mortality in birds induced from ascites and 20.33%, 6.67%, 13.33%, 6.67%, 0%, 13.33%, and 6.67% mortality induced from coccidiosis in control, T1, T2, T3, T4, T5, and T6 groups, respectively. The net return also revealed higher profit in treatment groups.

In experimental trial 4, at 42 day of age live body weight of chicken was found to be significantly increased in all treatment groups as compared to control group and highest body

weight was recorded in T3 group (supplemented with *P. armeniaca* extract @ 200 mg/kg body weight of chicken) followed by T4, T2, T1, T5, and T6. FCR value in the T3 group was found to be significantly improved among the groups. Highest mortality rate (20.00%, total 3 birds out of 15) was recorded in the control group birds followed by T1, T2, T4, and T6 (6.67%, total 1 out of 15). Interestingly, no mortality was recorded in T3 and T5 group during the entire period. Post-mortem examination revealed 13.30%, and 6.67% mortality in birds induced from ascites in control and T2 group respectively whereas 6.67% mortality was recorded each in control and T1 group induced from coccidiosis. Economics of the experiment was also calculated based on the rearing cost of 15 no of birds in each group. Due to lesser mortality higher profit was gained in treatment groups

Conclusion

The *in-vivo* efficacy of *R. imbricata, H. rhamnoides, S. alba,* and *P. armeniaca* extract was evaluated which indicated favorable changes in physio-biochemical indices, growth performance and survivability of broilers chickens. Further, cost economic of broiler rearing at high altitude indicated significant increase in farm income. The beneficial effect of these plant extracts on these parameters at different dose concentrations might be due to the synergistic effect of polyphenols, flavonoids, and carotenoids present in the extract. Efficacy study indicated that among the different dose level of phytoextracts, 400 mg/kg body weight of chicken of *R. imbricata*, 200 mg/kg body weight of chicken of *H. rhamnoides*, 300 mg/kg body weight of chicken of *S. alba*, and 200 mg/kg body weight of chicken of *P. armeniaca* has shown better effect as compared to other dose regime. Therefore, considering the beneficial effects of these phytoextracts, a new feed additive may be prepared/formulated using individual extracts in certain ration or preparation.

Objective 4: To formulate the phytogenic feed additive for broiler chicken and a comparative study on their efficacy with other commercially available feed additives.

Material and methods

In the present study, a new phytogenic feed additive was prepared and its efficacy was compared with commercially available feed additives for their effect on growth performance of broiler chickens at high altitude. To prepare the feed additive, aqueous extract of *H. rhamnoides* fruits, *S. alba* leaves and *P. armeniaca* seeds were mixed together and three formulations were prepared to have different concentration of extracts. The prepared formulations were F1 (contains, *H. rhamnoides* @200mg/kg body weight of chicken, *S. alba*

@200mg/kg body weight of chicken, and *P. armeniaca* @100mg/kg body weight of chicken), F2 (contains, *H. rhamnoides* @100mg/kg body weight of chicken, *S. alba* @300mg/kg body weight of chicken, and *P. armeniaca* @100mg/kg body weight of chicken), and F3 (contains, *H. rhamnoides* @100mg/kg body weight of chicken, *S. alba* @200mg/kg body weight of chicken, and *P. armeniaca* @200mg/kg body weight of chicken). Total antioxidant capacity, total phenolics, flavonoids and carotenoids content in the prepared formulations were analysed with previously described methods (objective 2).

Thereafter, efficacies of these prepared formulations were compared with available commercial feed additives. A total of 175 one day old broiler chicks were randomly assigned to seven groups in five replicates (5 chicks in each replicate) having 25 birds in each group. Experimental groups included control (fed the basal diet), T1 (basal diet + F1 formulation), T2 (basal diet + F2 formulation), T3 (basal diet + F3 formulation), T4 (basal diet + commercial probiotic), T5 (95 % basal diet + 5 % Lucerne powder), and T6 (basal diet + commercial herbal feed additive), respectively. Commercial broiler feed (SMPOORNA feed, Phagwara; starter code No. 08; finisher code No. 07) was used as basal diet in this study. Growth performance parameters were same as discussed earlier in the section (objective 1 and objective 3).

Results

The prepared feed additive formulations were found to be rich in the total antioxidant capacity as well as rich in phenolics, flavonoids and carotenoids content. Total antioxidant capacity in feed additive formulations was recorded to be 473.82 ± 18.11, 459.34 ± 19.07, and 455.11 ± 18.32 μM Fe (II)/g of extract in F1, F2, and F3, respectively. The content of total phenolic in feed additive formulations F1, F2, and F3 was recorded to be 269.16 ± 3.17, 241.26 ± 2.90, and 239.45 ± 3.05 mg GAE/g of extract, respectively. Similarly, the content of flavonoids in F1, F2, and F3 was recorded to be 94.67 ± 1.12, 88.14 ± 0.96, and 88.01 ± 1.15 mg QE/g of extract, respectively. Moreover, carotenoid content in F1, F2, and F3 was recorded to be 4.56 ± 0.32, 4.30 ± 0.27, and 4.34 ± 0.40 mg/100g extract, respectively. Total phenolic, flavonoid, and carotenoid content was higher in F1 formulation as compared to F2 and F3 formulations. The higher content of antioxidants in the feed additive formulations might be due to the synergistic effect of diverse phytomolecules found in *H. rhamnoides*, *S. alba*, and *P. armeniaca* extract which showed a more protecting effect against oxidative stress while acting in combination rather than individually [20].

At 21 day of age, birds in T2 & T3 groups that were supplemented with formulations F2 & F3 respectively showed significantly ($P<0.05$) higher body weight among the treatment groups. No differences were observed in average body weight among the control, T1 and T6 groups at 21 day. At 42 day of age, average body weight was increased in T1 (supplemented with formulation F1) group followed by T2 and T3 group. Moreover, no difference was recorded in the average body weight among the control, T4, T5, and T6 groups. Cumulative feed intake was decreased in T5 group as compared to other treatment groups. FCR value was significantly improved in T1 group. Highest mortality rate (16%, total 4 birds out of 25) was recorded in the control group birds followed by T5 and T6 (4%, total 1 out of 25). Interestingly, no mortality was recorded in T1, T2, T3 and T4 groups.

The increase in the body weight in treatment group birds that were supplemented with prepared feed formulation might possibly due to synergistic effect of active phytomolecules (gallic acid, kaempferol, salicin, caffeic acid, catechins, polyphenols, flavonoids, carotenoids) which can stimulate increased digestion and increased metabolism of nutrients causing higher efficiency in the utilization of feed and eliminates the production of free radicals which ultimately results in enhanced growth in birds [21-23]. Moreover, among the three formulated feed additives, birds supplemented with formulation F1 had better growth performance which might possibly due to appropriate dose concentration of integral plant extracts that could be sufficient to reduce the catabolic activities in broilers under oxidative stress conditions.

Conclusion

A phytogenic feed additive formulation has been developed for broilers comprising phytoextract of different parts of herbal plants grow in high altitude Himalaya, that are rich in bioactive phytomolecules having stress-ameliorating and antioxidant properties. Feed additive formulation was comparatively tested with commercially available feed additives and it was established that feed additive formulation 1 (*H. rhamnoides* @ 200mg/kg body weight of chicken, *S. alba* @ 200mg/kg body weight of chicken, and *P. armeniaca* @ 100mg/kg body weight of chicken) contained a considerable amount of diverse phytomolecules and therefore supplementation of this formulation to broilers improves their growth performance. As a whole, it can be concluded that this feed additive formulation could be an aid for poultry birds in the stressful environment of high altitude where there is marked deficiency of nutritious poultry feed and fodder.

Salient findings of the study

1. Identification of suitable broiler strains having more adaptability under hypoxic conditions at high altitude is more important to overcome unsatisfactory growth performance in Leh-Ladakh. Among the three broiler strains, RIR cross-bred has shown better growth and survivability, which may be due to less metabolic rate.

2. These RIR cross-bred performs as dual purpose breed, which are more hardy and grow better withought stress. Hence, dual purpose poultry birds and slow growing broiler strains/breeds would be more suitable to this region.

3. Therefore, efforts should be given on development of these kinds of poultry birds for Leh-Ladakh.

4. Commercially available probiotics has no beneficial effect on weight gain, however has reduced mortality rate at Leh-Ladakh.

5. Hence, before the selection of probiotics, more studies are required to know the dose, microbial population, and kinds of gut microflora species prevalent in this farming system. This could be helpful for weight gain and reducing the mortality.

6. Hydro-ethanolic extract of *Rhodiola imbricata* roots and aqueous extract of *Hippophae rhamnoides* fruits, S*alix alba* leaves and *Prunus armeniaca* seeds were phytochemically characterized which revealed rich in polyphenols, flavonoids, and carotenoids content. Moreover, *in-vitro* studies indicated enhanced antioxidative and cytoprotective activities of these phytoextracts on chicken peripheral blood lymphocytes. Therefore, considering the pharmacological antioxidant properties of these four extracts their nutraceutical value might be evaluated for their utilization in feed additive as performance enhancer.

7. The *in-vivo* efficacy of *R. imbricata*, *H. rhamnoides*, *S. alba*, and *P. armeniaca* extract was evaluated which indicated favorable changes in physio-biochemical indices, growth performance and survivability of broilers chickens. Further, cost economic of broiler rearing at high altitude indicated significant increase in farm income. The beneficial effect of these plant extracts on these parameters at different dose concentrations might be due to the synergistic effect of polyphenols, flavonoids, and carotenoids present in the extract. Efficacy study indicated that among the different dose level of phytoextracts, 400 mg/kg body weight

of chicken of *R. imbricata*, 200 mg/kg body weight of chicken of *H. rhamnoides*, 300 mg/kg body weight of chicken of *S. alba*, and 200 mg/kg body weight of chicken of *P. armeniaca* has shown better effect as compared to other dose regime. Therefore, considering the beneficial effects of these phytoextracts, a new feed additive may be prepared/formulated using individual extracts in certain ration or preparation.

8. Total three phytogenic feed additive formulation has been developed for broilers comprising extract parts of different herbal plants grow in high altitude Himalaya, that are rich in bioactive phytomolecules having stress-ameliorating and antioxidant properties. Feed additive formulation was comparatively tested with commercially available feed additives and it was established that feed additive formulation 1 (*H. rhamnoides* @ 200mg/kg body weight of chicken, *S. alba* @ 200mg/kg body weight of chicken, and *P. armeniaca* @ 100mg/kg body weight of chicken) contained a considerable amount of diverse phytomolecules and therefore supplementation of this formulation to broilers improves their growth performance. As a whole, it can be concluded that this feed additive formulation could be an aid for poultry birds in the stressful environment of high altitude where there is marked deficiency of nutritious poultry feed and fodder.

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